CHANGES IN WEIGHT AND WATER CONTENT DURING THE LIFE CYCLE OF THE WOOD-EATING BEETLE, PASSALUS CORNUTUS FABRICIUS

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Introduction

That relative humidity and water relations in general are important in the lives of insects has been well established. The literature on the subject is voluminous. Excellent reviews by Buxton (1932), Mellanby (1935), and Johnson (1942) discuss insect water relations from various points of view, such as saturation deficiency and longevity, evaporation of water, the humidity of the environment, etc. Others (e.g. Johnson, 1937; Slifer, 1938) have studied the mechanism and rate of water absorption in the insect egg. Ludwig (1931), in reviewing the literature on the subject of changes in weight and water content during metamorphosis of insects, calls attention to the fact that in different species entirely opposite phenomena may take place at corresponding stages in development. To give one example: Bodine and Orr (1925) observed that in Drosophila a marked *increase* in weight occurred on emergence of the adult, while Ludwig (1931) found that during the metamorphosis of the Japanese beetle from larva to adult there was a *loss* of weight amounting to half the maximum larval weight. In both cases the changes in weight were attributed mainly to changes in water content. Thus it would seem that no hard and fast rule can be laid down that will apply to all insects; the story must be worked out independently for different species living under different conditions.

In this paper an attempt is made to establish the normal weight and water content of a beetle, *Passalus cornutus,* at all stages in its life history from egg to adult under stable conditions. A study of the ranges of tolerance to humidity and temperature are left for a later paper. Among the beetles there are few, if any, records of the changes in water content of a species throughout its whole life cycle. Ludwig's (1931) studies of the weight changes and water relations of the Japanese beetle larva, pupa, and adult offer the most satisfactory comparison to the conditions found in Passalus, although the habitat and duration of the life cycle of the Japanese beetle and of Passalus differ markedly.

Several factors make Passalus a convenient insect for study: large size, abundance, ease in obtaining, large size of eggs, and short life history. Passalus is a wood-eating beetle which spends practically all of its life in decaying hardwood logs. The adult beetles make extensive tunnels in the logs; and here the eggs are laid, and larval, pupal, and adult life is lived. The eggs are often deposited in an offshoot of a tunnel, out of the way of the main line of traffic. Although not all eggs of one female are laid at the same time, all her eggs are laid in the same place in the runway. Always they are on, and surrounded by, an abundance of moist, finely chewed frass prepared by the adults. Passalus is a social beetle. The adults protect the developmental stages, prepare frass for the larvae, and build cocoons for the pupae. The developmental cycle is completed in a relatively short time for so large a beetle. In the vicinity of Durham, N. C., eggs are laid in late May or June and new adults appear in August.

Only hardwood logs that have a high percentage of moisture are inhabited. In fact, the whole life cycle must of necessity be completed in a moist environment. Data at hand indicate that eggs will not hatch nor larvae develop except when in contact with moisture. Even the adults cannot live at low humidities, and the adults are more hardy in this respect than are the eggs, larvae, and pupae.

Materials and Methods

Passalus eggs of known age were obtained in the laboratory in the following manner. Laying females were brought in from the woods and placed in finger bowls with some of the frass from the logs. One female was placed in each finger bowl. The dishes were covered and kept in a dark cabinet. Each morning the frass was examined for eggs. When first laid the eggs are bright red in color and are not difficult to distinguish from the frass. As Passalus seldom lays more than two eggs in a 24-hour period, and as all females brought in do not lay in the laboratory, it takes a great many beetles to obtain a sufficient supply of eggs for experimental purposes. The eggs were placed in petri dishes containing either moist filter paper or moist frass. Volumes of the eggs were determined by the formula $V = {}^4\!/_3\pi a^2 b$, where a equals the shorter semi-axis and b equals the longer semi-axis. The calculations are probably not absolute, for all eggs are not perfectly ovoid, but volumes computed by this formula are relative and give very satisfactory comparisons. Measurements of the eggs were made to the nearest tenth millimeter with a vernier caliper. In all, several thousand eggs have been used in various experiments over a period of several years.

All stages in development were kept in an insulated cabinet at 27° C. Larvae, prepupae, and pupae were kept in large covered dishes with an abundant supply of frass and moisture. Larvae of different ages were kept in separate dishes to prevent the larger larvae from injuring and eating the smaller. Adults were not kept with the developmental stages. All weighings were made on a chain-o-matic balance. To determine water content, from 15 to 25 eggs of each age group were dried in a vacuum oven at 98° C. until the weights became constant.

Observations

Changes in weight, volume, and water content of the eggs

Figure 1 shows graphically the close correlation between weight and volume of Passalus eggs at different age levels. The data for these curves were obtained from material collected during a single season and kept under similar conditions. About 350 eggs are represented. All were weighed and measured individually, some each day, others every other day. That the increase in weight is due to an increase in water content is clearly shown in the parallel curves of Figure 2. The eggs represented here are not the same as those used for plotting the curves of Figure 1 and were collected in a different season. In each age class 15 to 25 eggs were weighed collectively, dried, and reweighed. The curves indicate that there is a slight decrease in dry weight as development proceeds.

Passalus eggs are large as insect eggs go, and are ovoid in shape. When first laid they measure approximately 3.20 mm. × 2.50 mm. and increase in size to about 3.80 mm. × 3.20 mm. at time of hatching. Newly laid eggs weigh between 10.0 and 12.0 mgs.; at time of hatching between 18.0 and 21.0 mgs. There is of course considerable individual variation in size and weight of the eggs. The amount of water in the eggs increases from 7.25 mgs. in newly laid eggs to over 15 mgs. in those ready to hatch. In per cent the water content increases from 68 to 83. Weight, volume and water content do not show marked changes for the first five days of development; but following this initial period of relative stability, weight, volume, and water content increase rapidly and continue to do so up to the time of hatching, which at 27° C. requires 15 days. Changes in color accompany the changes in weight, volume, and water content. Newly laid eggs are bright red. This gradually turns to a reddish-brown during the first week of development, then to a brownish-green, and within a few days of hatching to dark green. The significance of the color changes has not been determined.

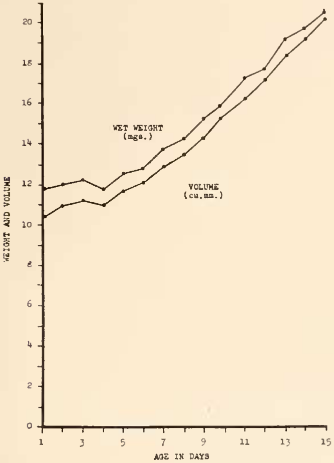

FIGURE 1. Changes in wet weight and volume during development of the eggs of *Passalus cornutus*.

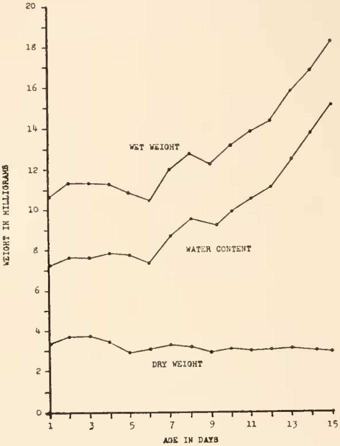

FIGURE 2. Changes in wet weight, dry weight, and water content during development of the eggs of *Passalus cornutus*.

Changes in weight and water content of larva and pupa

There are three larval instars. In the laboratory the first lasts about 16 days, the second about 13 days, and the third (including the prepupal stadium) about 28 days. Near the end of the third instar the larvae cease feeding and wander about for a few days seeking a place to pupate. Entrance upon the prepupal stadium is gradual and is determined with difficulty. As soon, however, as the prepupae become sluggish and take on their characteristic white, fatty appearance, they can then be readily distinguished from the active, bluish larvae that are still feeding. The prepupal stage lasts 5 to 6 days.

On hatching from the egg the larvae vary in length between 8.5 mm. and 11.5 mm. and weigh between 18.0 mg. and 23.0 mg. Little or no food is taken for the first day or so, but after feeding is well under way—in 24 to 36 hours—the color changes from white to bluish and the increase in size and weight is rapid. There is great individual variation, but the weight of a larva may increase to as much as 140 mg. by the end of the first instar, 400 mgs. by the end of the second instar, and 2000 mgs. by the end of the third instar. Thus the weight may increase a thousandfold in less than 2 months. The weight falls slightly after the late third instar larvae cease feeding and enter the prepupal stage; it rises slightly just before pupation begins; and again falls off, this time abruptly, on emergence of the adult. The weight of the adult is about five-sixths that of the greatest weight of the larva. Individual weight records were kept on many larvae raised in the laboratory and carried through to adulthood. A typical weight curve is given in Figure 3. Among the adults, as in all other stages, great individual variation in weight occurs. There is no sexual dimorphism, but the average female is larger and about 12 per cent heavier than the average male. The average weight of 27 old (not newly

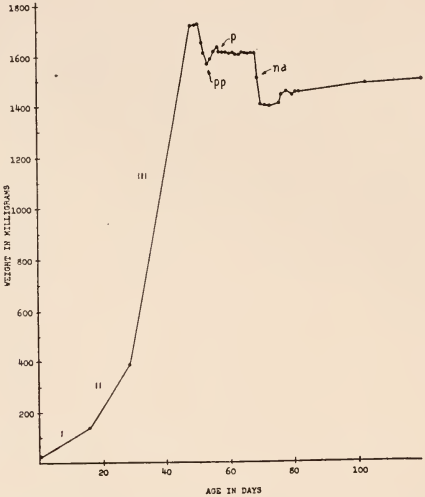

FIGURE 3. Changes in weight of *Passalus cornutus* from larva to adult. I, II, III, the three larval instars; pp, beginning of prepupal stage; p, beginning of pupal stage; na, newly transformed adult.

emerged) males was found to be 1478 mgs.; and of 35 old females, 1686 mgs. The range in weight of males was from 977 mgs. to 1740 mgs.; of females, from 1390 mgs. to 1972 mgs.

Newly hatched larvae contain about 85 per cent water. As soon as the larvae begin feeding the water content rises to about 90 per cent. This percentage is maintained until the larva ceases feeding and enters the prepupal stage when it falls to 88 per cent. There is then a steady increase in percentage of water until a high of 93 per cent is reached in the late pupal stage. The changes in water content in the prepupa coincide with the changes in weight, but in the pupa the weight remains constant while the percentage of water increases. A loss in dry weight is apparent as the internal tissues undergo histolysis. On emergence of the adult the per cent of water does not fall abruptly to the low level of old black adults, but declines gradually. The total weight of the adult, however, remains essentially constant so that the gradual decrease in percentage of water is explained by a gradual increase in dry weight. The red color of newly emerged adults persists for several weeks and may last for several months, but as the red gradually gives way to brown and the brown to black, the percentage of water falls until it finally reaches the level of 67 per cent for old adults. Twelve adults a month after emergence were found to still contain as much as 76.5 per cent water. No appreciable difference in the water content of males and females was observed.

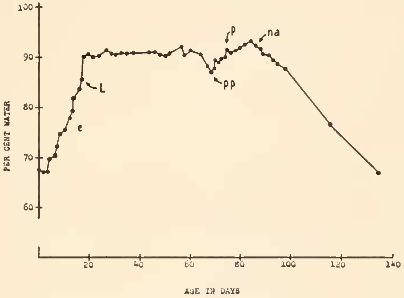

FIGURE 4. The water content, in per cent, throughout the life cycle of *Passalus cornutus*. e, egg; L, newly hatched larva; pp, beginning of prepupal stage; p, beginning of pupal stage; na, newly transformed adult.

A curve of the changes in percentage of water content from egg to adult is shown in Figure 4.

DISCUSSION

That Passalus eggs should absorb water is not surprising for this phenomen has been observed in other insect eggs. Bodine (1929) was among the first to show definitely that the increase in weight of an insect egg is due to an actual increase in water content. Slifer (1938) has given a brief history of our knowledge of this subject and in her own experimental work has demonstrated the manner in which water is absorbed by the grasshopper egg. She has shown that the ability to absorb water coincides with the formation of a special structure at the posterior end of the embryo. This she has designated as the hydropyle. In the eggs of *Melanoplus differentialis* there is no water absorption for the first 6 days of development (until the hydropyle has formed); then there follows a period of gradual increase in water content which lasts until several days before hatching, when the eggs become turgid and water absorption ceases. The shape of the weight curve presented by Johnson (1937) for Notostira indicates that the pattern of water absorption in the eggs of this insect is similar to that of the grasshopper: there is a lag of a few days followed by a rapid increase in weight (and water content); then a marked decline in the rate of increase occurs several days before hatching.

The curves of increase in wet weight and water content of Passalus eggs are similar to those of the grasshopper and Notostira except in one particular. In Passalus eggs water uptake begins about the fifth day, as in the grasshopper egg, but unlike either the grasshopper or Notostira the absorption of water continues up to time of hatching. The curve does not level off a few days before hatching time as in these other insects. If present at all, the "leveling-off period" must be very brief in Passalus eggs. Failure to demonstrate it cannot be explained by lack of data, for none of the many eggs, whose individual daily records of weight and volume were kept from time of laying to hatching, showed leveling-off in either weight or volume.

There is evidence to indicate that a hydropyle is formed in Passalus eggs. In fixed eggs a small ring is apparent at the posterior end of the egg about the fifth to sixth day of development, and its appearance can be correlated with the beginning of water uptake. Studies are in progress on the histological structure of the hydropyle of the Passalus egg.

As is to be expected the curve of egg volume parallels the curve of wet weight. The exact age of the egg cannot be determined by volume alone because of the large individual variations in the eggs. One can, however, estimate with a fair degree of accuracy whether an egg of unknown age is in its first, middle, or last third of development. Size and color together make fair criteria for roughly determining the ages of eggs found in logs.

The eggs practically double in wet weight, volume, and amount of water during their development. The contentions of Bodine (1929) and of Johnson (1937) that the gain in weight is due to water uptake are supported. Johnson (1937) has suggested that there is actually a decrease in dry weight of the eggs as development proceeds. It seems probable that this may be true, for in Passalus eggs there is a slight but noticeable downward trend in dry weight. However, at least in Passalus, a larger number of eggs should be used before drawing a definite conclusion on this point. The loss in dry weight is gradual and is probably caused by the transformation of the large amount of yolk into food materials and protoplasm.

In Passalus, while the curves of weight and water content of larvae, prepupae, and pupae follow the same trend as found by Ludwig (1931) for the Japanese beetle, some deviations worthy of mention occur. The time spent in larval stadia is much shorter in Passalus and growth is much greater and much faster. It is not uncommon for larvae to make a 10,000 per cent increase in weight in less than 2 months. Evidence indicates that under natural conditions in the logs in the forest, where temperatures often rise above laboratory temperatures, growth is even faster than reported in this paper. The water content of the larva is definitely higher in Passalus. It averages about 90 per cent in contrast to 78 per cent in the Japanese beetle larva. In both larvae a drop in weight occurs in the prepupal stage. In Passalus, however, the fall in weight begins as soon as the larva stops feeding and before it definitely enters the prepupal stage and is followed by an increase in weight before the pupal stage is reached. The increase in per cent of water in the prepupa and the pupa is readily accounted for by the degenerative changes of the larval tissues which take place in these stages during metamorphosis. The pupae, especially, are extremely delicate and in their natural runways would not last long were it not for the heavy protecting cocoon built around each by adults. In the late pupa the water content reaches a high of 93 per cent. This is above that of the larva. In both the Japanese beetle and Passalus a sharp drop in weight is apparent on emergence of the adult. In Passalus, however, the decline in weight is not nearly so great as in the Japanese beetle and sometimes amounts to no more than one-eighth the maximal larval weight, although usually it is about one-sixth. This is in marked contrast to the Japanese beetle, whose loss in weight amounts to one-half the maximum larval weight. Evidently the decline in the per cent of water in newly emerged adults of Passalus is more gradual, and coincides with the gradual increase in dry weight. Sclerotization is slow. New adults are very soft and remain within the cocoon for several days before venturing forth to compete with other members of the colony. The slow increase in dry weight is correlated with the slow and gradual changes in color and in degree of sclerotization that the adults undergo. The change from pink to red to brown to black usually requires a month or more, but may require several months. Eventually the beetle becomes black. When it does so the water content has reached the level of 67 per cent common to the old adults and the integument has become thick and hard. Thus, in percentage, the water content of the adult Passalus eventually gets back to its starting point in the egg.

SUMMARY

The changes in weight and water content from egg to adult have been shown for the wood-eating beetle, *Passalus cornutus*.

In the egg little change occurs for the first 5 days of development; then the weight of the egg, the volume of the egg, and the amount of water increase steadily until hatching. The increase in weight is due to water uptake. As weight, volume, and water content increase the color of the egg changes from bright red to dark green.

During larval life, which in the laboratory at 27° C. lasts approximately two months, the weight may increase more than 10,000 per cent. A slight fall in weight occurs when the larva ceases to feed and enters the prepupal stage. On emergence of the adult there is a sharp drop in weight amounting to about one-sixth the maximum larval weight.

The water content of a newly laid egg is about 68 per cent. This increases to 83 per cent at time of hatching. Newly hatched larvae contain about 85 per cent water, but after feeding begins this increases to about 90 per cent. A slight fall in percentage of water occurs when the larva ceases to feed, but increases again in the

prepupal and pupal stages until, in the late pupa, it reaches a high of 93 per cent. The fall in per cent water is gradual in newly transformed adults and several months may be required for it to reach the level of 67 per cent characteristic of old adults.

The gradual change in water content of the new adults is correlated with the slow increase in dry weight, slow sclerotization of the integument, and with changes in color from red to black.

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